Identification of Muscle-Type Carnitine Palmitoyltransferase I and Characterization of Its Atypical Gene Structure

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Received July 7, 2004

INTRODUCTION

In mammals, two kinds of adipose tissue exist: white and brown adipose tissues (WAT and BAT, respectively). WAT is known as an ordinary adipose tissue widely distributed in the body and its major role is the storage of bioenergy in the form of fat. BAT is an adipose tissue observed only in restricted parts of the body such as the interscapular and perirenal regions. In addition, the physiologic role of BAT is unique and different from that of WAT. Although brown adipocytes in BAT also contain fat droplets as do white adipocytes in WAT, brown adipocytes expend excess energy more as heat rather than store it as fat. This heat production, also called as nonshivering thermogenesis, is important in the maintenance of body temperature at birth, in the arousal stage of hibernation, and when animals are exposed to a cold environment. The efficient thermogenesis of BAT is attributed to high activity in the oxidation of respiratory substrates such as fatty acids and glucose in its mitochondria, with low activity for oxidative phosphorylation. Therefore BAT is thought to prevent mammals from excess storage of energy.1,2) This function of BAT is mainly supported by uncoupling protein-1 (UCP-1) present in its mitochondrial inner membrane.3–9) UCP-1 is specifically expressed in BAT and acts as a H+ conductor across the H+-impermeable mitochondrial inner membrane, as shown in Fig. 1. The proton-conducting action of UCP-1 results in dissipation of the proton motive force (Δ\(\mu\)H\(^+\)), like protonophoric uncouplers of oxidative phosphorylation.10,11) As a result, the excess energy in BAT is released in the form of heat without synthesis of ATP. Thus, although both BAT and WAT are adipose tissues, the function of BAT is unique.

Since UCP-1 plays a central role in the energy-dissipating reaction in BAT, the mechanisms controlling its expression and activity have been investigated in great detail. However, it appears difficult to explain all of the functional differences between these two adipose tissues solely based on the presence or absence of UCP-1, and we hypothesized that there could be some biological system(s) other than UCP to support the unique function of BAT. Therefore we attempted to identify a protein(s) specifically expressed in BAT but not in WAT by comparing the messages existing in these two adipose tissues.

ISOILATION OF A NOVEL cDNA CLONE DS112 FROM RAT BAT

To compare the messages existing in BAT and WAT, we carried out differential screening.12) A cDNA library of rat BAT, constructed in a λZAP phage, was prepared with poly(A)+ RNA purified from interscapular BAT from 4-week-old male Wistar rats. The phages were allowed to infect the host bacteria, and then the bacteria were plated onto agar plates. The probe DNA for library screening was prepared by reverse-transcription of poly(A)+ RNA from rat epididymal WAT with an oligo (dT) primer.

Differential screening was performed according to the general procedure with slight modification as follows. First, the phage DNAs containing cDNA of rat BAT on agar plates were transferred to nitrocellulose membranes for screening. The DNAs of phages on these membranes were hybridized with radiolabeled cDNA probes of WAT and UCP-1 (the cDNA library of rat BAT was screened with the probe for WAT and UCP-1). After autoradiography, we chose 200 phages of which the DNA did not appear to be hybridized with the cDNA probes for WAT and UCP-1. Then the 200 cDNA clones were further analyzed with Southern blotting with cDNA probes for both BAT and WAT. Consequently, we obtained one cDNA clone, DS112, that hybridized with the probe for BAT but not with the probe for WAT. Upon...
digestion with restriction endonucleases, the length of the cDNA (DS112) was shown to be about 1.5 kbp. Next, to confirm whether the transcript corresponding to DS112 actually existed in BAT but not in WAT, Northern blot analysis with the cDNA probe for DS112 was performed. A large amount of transcript hybridizing with the cDNA probe of DS112 was observed in an RNA sample from BAT, but an almost negligible amount was seen in the sample from WAT.

**DS112 ENCODES A NOVEL PROTEIN RELATED TO MITOCHONDRIAL CARNITINE PALMITOYLTRANSFERASE I**

From the results of Northern blot analysis, the length of the transcript corresponding to DS112 was estimated to be about 3 kb, indicating that the isolated clone DS112 (1.5 kbp) did not contain the full-length DNA sequence. We carried out further screening of the rat BAT cDNA library with the cDNA of DS112 as a probe and obtained another clone, DS112-36. From the nucleotide sequence of DS112-36, one open-reading frame (ORF) spanning 2316 bp was found to be encoding an 88.2-kDa protein consisting of 772 amino acids. The putative translation initiation codon was maintained with the Kozak consensus sequence.13)

In a homology search using BLAST,14) we found no entry with completely or almost the same nucleotide sequence as that of DS112-36 in all species. Hence cDNA clone DS112-36 was found to encode a novel protein. However, the nucleotide sequence of DS112-36 showed the highest homology of about 60% with that of carnitine palmitoyltransferase I (CPTI) from rat liver, an 88.1-kDa protein consisting of 772 amino acids. The putative translation initiation codon was maintained with the Kozak consensus sequence.15)

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**MITOCHONDRIAL CARNITINE PALMITOYLTRANSFERASE I**

In mammalian cells, most long-chain fatty acids are degraded via β-oxidation in the mitochondrial matrix space. For this process, long-chain fatty acids are converted to acyl-CoAs by acyl-CoA synthetase in the cellular cytosolic space (extramitochondrial space) and transported into the mitochondrial matrix space in the form of acyl-CoAs. However, as the inner membrane is not permeable to long-chain fatty acyl-CoAs, they penetrate into the matrix space via a “carnitine system” consisting of the following three components: 1) formation of acylcarnitine from long-chain fatty acyl-CoAs catalyzed by CPTI located in the mitochondrial outer membrane; 2) import of acylcarnitine in exchange with free carnitine in the matrix space mediated by a carnitine-acylcarnitine carrier located in the inner membrane; and 3) formation of long-chain fatty acyl-CoAs from the imported acylcarnitines catalyzed by CPTII located on the inner side of the inner membrane (Fig. 3). Of these three proteins, the activity of CPTI is inhibited by a simple molecule, malonyl-CoA, at a physiologic concentration. Malonyl-CoA is synthesized from acetyl-CoA by acetyl-CoA carboxylase and is the first committed intermediate in the pathway of fatty acid synthesis. That is, the catalytic activity of CPTI is regulated by the state of mitochondrial fatty acid synthesis and appears to determine the rate of fatty acid transport to the mitochondrial matrix space. Because CPTI is responsible for the first rate-limiting step in the oxidation of fatty acids in mitochondria, considerable attention has been paid to its structural and functional features.15—19)

About 20 years ago, kinetic analysis of CPTI from rat liver, heart, and skeletal muscle mitochondria revealed $K_m$ values for carnitine of ~30, 200, and 500 μM, respectively, and IC$_{50}$ values for malonyl-CoA (the concentration needed to inhibit activity by 50%) of ~2.7, 0.1, and 0.03 μM, respectively.20) These observations suggested that there might be more than one isoform of CPTI in rat tissues. Later, using a radioactive covalent ligand for CPTI, it was shown that the major protein labeled in rat heart and skeletal muscle had an...
**Fig. 2. Comparison of a Protein Encoded by a Novel cDNA Clone DS112-36 with Rat Liver CPTI**

A) Alignment of the predicted amino acid sequence of DS112-36 with that of rat liver CPTI. Asterisks indicate identical residues. Four gaps shown by (---) are inserted to obtain maximum alignment.

B) Hydropathy profiles of DS112-36 and rat liver CPTI. Amino acid sequences were analyzed by the method of Kyte and Doolittle with an interval of 30 amino acids.

**Fig. 3. Mitochondrial Carnitine System**
apparent molecular size of 82 kDa and it was clearly separable on SDS/polyacrylamide gels from the labeled CPTI in rat liver (88 kDa). By using anti-rat liver CPTI antibody, it was also suggested that rat heart, skeletal muscle, and BAT mitochondria contain a distinct protein from rat liver enzyme. Finally, in 1993, a cDNA clone encoding CPTI was isolated from a rat liver cDNA library. From the nucleotide sequence, the molecular weight of L-CPTI was estimated to be 88 kDa. Northern blot analysis using the cDNA probe for rat L-CPTI showed that a 4.7-kb message of L-CPTI was detected in the liver and heart but not in skeletal muscle. Moreover, using a selective inhibitor of L-CPTI, it was shown that the liver-type isofrm contributes ~2—3% to the total CPTI activity in the heart and the remaining CPTI activity is contributed by the muscle-type isofrm (M-CPTI).

Based on the previous observations described above, it has been suggested that there are at least two forms of CPTI, L- and M-CPTI, in mammalian cells and that both types of CPTI are expressed in the heart. However, as the isolation and purification of the CPTI protein were difficult, the nucleotide or amino acid sequences of M-CPTI have not been reported, and the molecular analysis of M-CPTI has not been performed.

TISSUE DISTRIBUTION OF THE TRANSCRIPT OF DS112

To confirm whether DS112 (DS112-36) isolated from rat BAT encodes M-CPTI, we further examined the tissue distribution of the transcript of DS112-36 using Northern blotting. As shown in Fig. 4, the strong signals of the transcript hybridizing with the probe of DS112-36 were observed not only in BAT, but also in heart and skeletal muscle, with a weak signal in WAT and a faint signal in the kidney. On the other hand, the transcript for L-CPTI was observed mainly in the liver and also in the kidney and WAT, while weak signals were detected in the brain and heart. The transcript level of rat CPTII was almost identical in all tissues examined except for the brain.

As described above, a protein encoded by the novel cDNA clone DS112-36 from rat BAT was shown to be very similar in structural features to rat L-CPTI, and the transcript corresponding to DS112-36 was mainly expressed in BAT, heart, and skeletal muscle. We therefore concluded that DS112-36 could encode M-CPTI, a novel isoform of L-CPTI. An expression experiment in mammalian cells revealed that the protein encoded by this cDNA has physical and kinetic characteristics identical to those of CPTI in muscle mitochondria. In addition, although rat M-CPTI was thought to be an 82-kDa protein based on its electrophoretic mobility, the size estimated based on the nucleotide sequence of isolated cDNA (DS112-36) was about 88 kDa. This anomalous electrophoretic mobility of rat M-CPTI was shown to be due to its primary structure (not due to the posttranslational modification) in in vitro transcription and translation experiments.
muscle, like rat M-CPTI. It is noteworthy that M-CPTI is expressed in cells that consume bioenergy extensively by using fatty acids as their major respiratory substrate. In addition, the human M-CPTI gene was reported to be located on chromosome 22q13.3.33,34) and peroxisome proliferators and a fatty acid response element was located on the 5'-upstream end of exon 1A of the human M-CPTI gene (Fig. 5).35—37)

TRANSCRIPTS CONTAINING BOTH REGIONS OF THE HUMAN CK/EK-β AND M-CPTI GENES

Although we characterized the gene structure of human M-CPTI as described above, we did not determine the exact transcription initiation site(s) of the gene. Subsequently, another group reported two transcription initiation sites of human M-CPTI.37) Of those two sites, although one (TSS M) was coincident with the 5'-end of exon 1B as determined by us, the other one (TSS U) was located about 40 bp downstream from the 5'-end of exon 1A (Fig. 6A). This result suggested that there is a transcription initiation site(s) other than the two reported sites (TSS U and TSS M).

Therefore, to characterize the more detailed structure of the 5'-upstream region of the human M-CPTI gene, we carried out single-strand (ss) ligation to ss-cDNA-PCR (SLIC-PCR), a modified method of 5'-RACE. As a result, one cDNA clone, HHR-A10 from human heart mRNA, was shown to have a strange nucleotide sequence. HHR-A10 contained the nucleotide sequence of exon 1A, but nucleotides unexpected from the gene structure of M-CPTI were added to its 5'-end, as shown in Fig. 6B. By comparison
of the nucleotide sequence of HHR-A10 with that of genomic DNA, the unexpected nucleotides added to the 5'-end of HHR-A10 were suggested to be derived from the 3'-untranslated region (3'-UTR) of the CK/EK-b gene, and this unexpected sequence was linked to exon 1A, conforming to the GT-AG rule of the intron/exon boundary. These results suggest that the cDNA clone HHR-A10 is derived from the mRNA, in which the 3'-UTR of the CK/EK-b gene is directly linked to the third cytosine base of exon 1A of the human M-CPTI gene by splicing.

To confirm whether such a transcript is actually present in human tissues, RT-PCR between both exons of the CK/EK-b and M-CPTI genes was performed (Fig. 7A). As shown in Fig. 7B, amplified products were detected in mRNA of human heart and skeletal muscle. Based on their nucleotide sequences, PCR product 1 showing slower migration was shown to contain the 3'-UTR of CK/EK-b gene and exons 1A, 2, and 3 of the M-CPTI gene without exon 1B. Again, the first two nucleotides of exon 1A were deleted according to the GT-AG rule as in HHR-A10. However, product 2 showing faster migration contained the same CK/EK-b region as that of product 1, and its 3'-end correctly linked to exon 2 of the M-CPTI gene without exon 1A, conforming to the GT-AG rule. These results clearly show that the transcripts containing both regions of these two genes existed in human heart and skeletal muscle in considerable amounts.

Moreover, using Southern blot analysis, we found that there was no other region homologous to the CK/EK-b gene in the entire human genome. Thus such a transcript(s) containing both regions of the two genes was concluded to be produced from the functional genes of CK/EK-b and M-CPTI in human tissues.

CHARACTERIZATION OF THE TRANSCRIPTS BETWEEN CK/EK-b AND M-CPTI GENES

As shown in Fig. 8, at least four possibilities could be considered for the formation of the transcripts containing both regions of the CK/EK-b and M-CPTI genes: A) incomplete termination of the CK/EK-b message, in which transcription does not terminate at the 3'-end of the CK/EK-b
gene but proceeds to a specific region of the M-CPTI gene and the poly(A) tail is added; B) transcription from a specific region of the CK/EK-β gene occurs through the 3' end of the M-CPTI gene; C) transcription is initiated in a specific region of the CK/EK-β gene and termination occurs in a specific region of the M-CPTI gene; and D) transcription is initiated from the transcription initiation site of the CK/EK-β gene to the 3' end of the M-CPTI gene.

Since the existence of such transcripts containing the regions of the two genes was predicted from the nucleotide sequences of partially amplified cDNAs (obtained by 5'RACE and/or by RT-PCR between exon 11 of the CK/EK-β gene and exon 3 of the M-CPTI gene), the exact organization of these transcripts was still unclear. Therefore we further performed RT-PCR experiments using ss-cDNA obtained by reverse-transcription with specific oligonucleotides for the M-CPTI message. As schematically shown in Fig. 9A, the messages from human heart were specifically reverse-transcribed with the primer EX4, EX15, or EX20, which correspond to exon 4, 15, or 20 of the human M-CPTI gene, respectively. Then the ss-cDNAs obtained were used as templates for PCR with various primer pairs. The reaction mixtures were subjected to agarose gel electrophoresis and stained with ethidium bromide.

As shown in Fig. 9B, when ss-cDNA obtained by reverse-transcription of mRNA with primer EX4, referred to as EX4 ss-cDNA, was used as a template for PCR, a significant band was observed in amplification with primer pairs of 1A/EX3 and 1B/EX3. The nucleotide sequences of each amplified product showed that they were derived from ordinary transcripts of M-CPTI containing exon 1A or exon 1B, respectively. In contrast, two bands, a significant band with lower electrophoretic mobility and a weak band with higher mobility, were detected with the primer pairs of 9/EX3, 10/EX3, and 11/EX3. Sequence analyses showed that the products with the lower mobility band contained exons of the CK/EK-β gene and exons 1A, 2, and 3 of the M-CPTI gene, and products with the higher mobility band were the same as those with the lower mobility band except that exon 1A of the M-CPTI gene was not present. In these products, the CK/EK-β region was connected to the M-CPTI region in manners similar to those shown in Figs. 6 and 7. Using ss-cDNA prepared by reverse-transcription with EX15 (EX15 ss-cDNA) as a template, similar electrophoretic bands were observed with all primer pairs except for their intensities.

On the contrary, using EX20 ss-cDNA as a template, the products amplified by PCR between the two genes (with primer pairs of 9/EX3, 10/EX3, and 11/EX3) could not be detected by staining with ethidium bromide, although the PCR products of the M-CPTI region were observed, similar to the results with EX4 and EX15 ss-cDNA (with primer pairs of 1A/EX3 and 1B/EX3). Thus the PCR products in gel were transferred to the membrane and Southern blot analysis with specific probes was carried out. As a result, PCR products between the CK/EK-β and M-CPTI genes were also detected by using EX20 ss-cDNA as a template (Fig. 9C). The sizes of each product hybridized with the probe appeared to be the same as those obtained with EX4 and EX15 ss-cDNAs. Sequence analysis of the products amplified by the primer pair of 9/EX3 with EX20 ss-cDNA showed that they contained the same nucleotides as those obtained with EX4 and EX15 ss-cDNAs.

The products of the primer pair of 9/EX3 with EX20 ss-cDNA should be derived from the transcripts containing the nucleotides between primer 9 (exon 9 of the CK/EK-β gene) and primer EX20 (exon 20 of the M-CPTI gene). Thus the message containing the region from exon 9 of the CK/EK-β gene to exon 20 of the M-CPTI gene was the longest message actually detected in the present study.
Because the primer EX20 used for reverse-transcription corresponds to the more downstream region of the translation termination codon for the M-CPTI gene, the organization of the overlapping transcripts between the two genes predicted the possibilities B or D in Fig. 8. The existence of such overlapping transcripts containing the regions of the CK/EK-b and M-CPTI genes in human cells was confirmed by another group.39)

In the overlapping transcripts containing exon 1A of human M-CPTI, the first two nucleotides, adenine and guanine of exon 1A, were always excluded because of splicing according to the GT-AG rule (Fig. 6B). Therefore it is possible that the cDNA, which was initiated from the 5'-end of exon 1A as obtained by 5'-RACE, was not derived from the overlapping transcripts but from a transcript initiated at the 5'-end or a more upstream region of exon 1A of the M-CPTI gene.

CHARACTERIZATION OF THE TRANSCRIPTS CONTAINING BOTH REGIONS OF THE CK/EK-b AND M-CPTI GENES IN RODENTS

To examine whether there was an overlapping transcript(s) containing the regions of both the CK/EK-b and M-CPTI genes in other species, we performed RT-PCR experiments using rat and mouse ss-cDNAs as templates. The samples of poly(A)+ RNA of rat and mouse heart were reverse-transcribed, and the ss-cDNAs obtained were utilized to amplify the region between exon 11 of the CK/EK-b gene and exon 3 of the M-CPTI gene (Fig. 10A). As shown in Fig. 10B, two major products (products 1 and 2) were detected in rodent as well as in human heart. Sequence analysis showed that these products from rodent heart also contained both the CK/EK-b and M-CPTI regions, and the regions of two genes were bound by the GT-AG rule for intron splicing, although their organization was not completely the same as those in humans. In rodents, the shorter product (product 2) contained exon 11 of the CK/EK-b gene and exons 2 and 3 of the M-CPTI gene were similar to the shorter one in humans. The longer one (product 1) contained more of the downstream region of the 3'-end of the CK/EK-b gene and the CK/EK-b region connected to the 5'-end of exon 2 of the M-CPTI gene. In any product, the 5'- and 3'-ends of exon 2 and the 5'-end of exon 3 of the M-CPTI gene were completely identical to those of ordinary transcripts of M-CPTI. However, in rodents, it appears that there is no transcript containing the region corresponding to exon 1A of the human M-CPTI gene. In addition, the amounts of such overlapping transcripts observed in rodents were much lower than those in humans. In human cells, each PCR was performed in 25 μl of reaction mixture, and only 5 μl of mixture was loaded onto agarose gel and stained with ethidium bromide. However, in experiments in mouse or rat cells, all reaction mixtures in two or eight reaction tubes, respectively, were necessary to detect the amplified products (Fig. 10B).

Thus, to examine the relationship between the amount of the ordinary transcripts of CK/EK-b and that of the overlapping transcripts containing the regions of the two genes, we carried out Northern blot analysis of CK/EK-b. As shown in Fig. 11, the steady-state transcript levels of rodent CK/EK-b were not very much lower than those detected in human tissues, as reported previously.40) We therefore concluded that the amount of the transcripts containing both the CK/EK-b and M-CPTI regions did not depend on the transcript level of CK/EK-b.41)
POSSIBLE ORGANIZATION AND PRODUCTION MECHANISM OF TRANSCRIPTS CONTAINING BOTH REGIONS OF THE CK/EK-β AND M-CPTI GENES

We found that there are unordinary transcripts containing the regions of CK/EK-β and M-CPTI genes in addition to each ordinary message in mammalian cells, and such transcripts contain at least the entire ORF of M-CPTI, although the initiation site(s) is unknown. The genes encoding CK/EK-β and M-CPTI thus appear to overlap. Overlapping genes are commonly observed in prokaryotes, bacteriophages, viruses, and in organelles such as mitochondria, because the sizes of those genomes are relatively small. In addition, it is well known that overlapping genes in those organisms often form operons. In many cases, operons contain genes that are functionally related. Because eukaryotes like mammalian cells have relatively large genomes, it was believed that genes are randomly (uniformity) arranged in large genomes and that overlapping genes occurred much less frequently in eukaryotic nuclear genomes. However, since overlapping genes in mammalian genomes were first identified in mice, many cases of tight clustering and/or overlapping genes have been reported, although they are rare.

For example, the genes of the myosin I heavy chain-like protein and preprotachykinin B are located close together with a tail-to-head formation, similar to the case of the CK/EK-β and M-CPTI genes, and the nucleotides in a specific region are included in both messages. However, the splice sites in this overlapping region are not identical in each gene, resulting in exons of the two genes in an overlapping region having nucleotides different from each other. Whereas, in the case of the CK/EK-β and M-CPTI genes, the transcripts containing the two-gene region appear to be produced by splicing at exactly the same position as each ordinary messages, except for the boundary site of the two regions (the 3’-end of the CK/EK-β region and the 5’-end of the M-CPTI region in overlapping transcripts are not used as splice sites in ordinary messages).

We thus deduced that the production mechanism of such transcripts is as follows: Because the CK/EK-β and M-CPTI genes are tightly located with the same strand direction, transcripts initiated from the inside of the CK/EK-β region (or from the transcription initiation site of the CK/EK-β gene) proceed to the M-CPTI region. On this transcript, before the addition of the poly(A) tail at the ordinary site of the CK/EK-β region, a splice reaction could occur between the CK/EK-β and M-CPTI regions at the sites shown in Figs. 7 and 10. After that, transcription continues to the 3’-end of the M-CPTI region, the splice reaction occurs at ordinary sites of both gene regions, and the poly(A) tail is added at the ordinary site of the M-CPTI region.

In addition, there are at least two possible explanations for the species differences in the amount of such transcripts (Fig. 10). First, such transcripts are initiated inside the CK/EK-β gene via an unidentified promoter (possibility B in Fig. 8), and the promoter activity of the human gene is much higher than those of the rodent genes. In this case, there should be a gene encoding a protein lacking the amino-terminal region of CK/EK-β or a protein having a completely new sequence, in addition to the ordinary CK/EK-β and M-CPTI genes. Second, the transcripts containing the regions of both genes begin from the initiation site of the CK/EK-β gene (possibility D in Fig. 8), but the termination of transcription and/or the addition of the poly(A) tail to the primary transcript of CK/EK-β would occur more easily in rodents than in humans, or the splicing between the regions of the two genes in the primary transcripts is more difficult in rodents than in humans. In this case, a message like the polycistronic transcript containing complete ORFs of CK/EK-β and M-CPTI should be produced.

CONCLUSIONS

A cDNA encoding rat M-CPTI was isolated as a novel cDNA clone by differential screening of a rat BAT cDNA library with a cDNA probe for WAT. Northern blot analysis showed that M-CPTI is dominantly expressed in cells that consume bioenergy extensively by using fatty acids as their major respiratory substrate. The gene structure of human M-CPTI was characterized and the gene for CK/EK-β was shown to be located only about 300 bp upstream from the M-CPTI gene with the same strand direction. It appears that there is no functional relationship between CK/EK-β and M-CPTI.

Upon further structural analysis, we found that there were unordinary transcripts containing both regions of the CK/EK-β and M-CPTI genes in addition to each ordinary transcript. With such a proximate organization of the two genes, the transcription of the CK/EK-β gene may proceed to the M-CPTI gene, and splicing may occur between the two genes before addition of the poly(A) tail. Therefore the existence of a transcript containing the region from the initiation site of CK/EK-β to the 3’-end of M-CPTI, in which the 3’-UTR of CK/EK-β gene is directly linked to the 5’-UTR of M-CPTI gene, was suggested. Until now, the physiologic roles of such transcripts containing both CK/EK-β and M-CPTI were unknown. However, the fact that such transcripts were produced from two functionally unrelated genes is interesting.

It has been reported there is an element responsible for peroxisome proliferators and fatty acids on the 5’-region of the human M-CPTI gene and that those molecules regulate the transcription of the M-CPTI gene. Moreover, because the CK/EK-β gene is located close to the 5’-terminal of the
M-CPTI gene, there might be another transcriptional regulation element(s) for the M-CPTI gene within the CK/EK gene. If so, the state of the CK/EK-b gene (transcriptional activate state or inactivated state) might regulate the transcription of the M-CPTI gene. The control mechanism of gene expression of M-CPTI as well as the physiologic roles of the overlapping transcripts should be analyzed in future studies.

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